UNIVERSITY OF PENNSYLVANIA

VARIABILITY OF POLYPORUS SCHWEINITZII IN CULTURE

THOMAS WHITE CHILDS

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

PHILADELPHIA
1936

VARIABILITY OF POLYPORUS SCHWEINITZII IN CULTURE

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL OF THE UNIVERSITY OF PENNSYLVANIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

THOMAS WHITE CHILDS

PHILADELPHIA
1936

R. UNIV.
BIBLIOTHEEK
LEIDEN

[Reprinted from PHYTOPATHOLOGY, January, 1937, Vol. XXVII, No. 1, pp. 29–50.]

VARIABILITY OF POLYPORUS SCHWEINITZII IN CULTURE[1]

THOMAS W. CHILDS[2]

(Accepted for publication May 1, 1936)

INTRODUCTION

Slight cultural differences within various species of wood-attacking fungi have been briefly mentioned by Long and Harsch (8) and by Fritz (3), but these workers, primarily interested in the diagnosis of decay, attached little importance to the variability they encountered. Schmitz (14) has described definite morphological and physiological differences between 4 isolations of *Fomes pinicola* (Sw.) Cke. Mounce (11), also working with *F. pinicola*, has discussed differences between cultures from a number of sources and ascribes these differences to individual variation rather than host influence. Aside from these studies, intraspecific variability in the Polyporaceae seems to have received little or no detailed attention, although the possibility of its occurrence often has been suggested.

When *Polyporus schweinitzii* Fr. was found causing a destructive root rot in 20- to 25-year-old plantings of northern white pine, *Pinus strobus* L., on unfavorable sites near Springwater, New York,[3] the possible implication of a heretofore unreported parasitic strain of either native or foreign origin could not be ignored. Cultures of this fungus, therefore, were requested from several American and foreign sources, and a number of isolations were made from diseased white pines at Springwater. A few isolations also were made from white pine and larch in New England. As this material accumulated it became increasingly apparent that there was no morphological basis for subdividing the species into local, parasitic, or host-specialized strains. Some of the cultures resembled each other rather closely, and all of them had a few characters in common, but with the exception of those taken from within a few feet of each other no two were identical, nor did those from any given host or locality manifest any special similarity.

In the past, *Polyporus schweinitzii* has been known principally as a cause of heart rot in butts of over-mature trees; with the disappearance of

[1] Presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] This study was begun during the tenure of a Morris Arboretum Fellowship in the Department of Botany of the University of Pennsylvania, and was completed at the Morris Arboretum with the assistance of a fellowship granted by the Charles Lathrop Pack Forest Education Board. The writer also wishes to acknowledge his indebtedness to Dr. H. H. York and Dr. Conway Zirkle, to Edith Adams Childs for laboratory assistance, and to the many workers who furnished cultures.

[3] York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa*. Unpublished manuscript.

virgin stands it was expected to become relatively unimportant. Its destructiveness at Springwater and in a Douglas fir plantation near Biltmore, North Carolina, (6) suggests, however, that it may constitute a serious obstacle to the use of coniferous species in artificial reforestation, at least on certain sites. An investigation of its variability has, therefore, been undertaken to determine to what extent specialized strains may be responsible for damage to young trees. Certain preliminary observations are presented here. Final conclusions will be possible only when the results of field studies, already inaugurated, become available.

SOURCES AND DESCRIPTIONS OF CULTURES

It was the writer's practice to make several isolations from each sporophore or infected tree from which a culture of the fungus was desired. More than 100 isolations, representing 25 sporophores and 9 trees, were thus secured and cultured on nutrient agar under closely comparable conditions. With 2 exceptions, isolations from any given tree or sporophore were indistinguishable from one another, but differed more or less distinctly from those from any other source. It, therefore, was assumed that similar isolations from the same source were derived from the same individual mycelium, *i.e.*, were members of the same clone, and that dissimilar isolations were derived from genetically different mycelia. These mycelia, together with those obtained from other workers, will be referred to by the following numbers:

1 to 30, inclusive. From *Pinus strobus*; Springwater, N. Y.
31. From *Pinus strobus* (lesion on 0.5 cm. root of an 8-year-old planted tree); Honeoye Lake, N. Y.
32. " " " ; Weston, Ontario.
33. " " " ; Lake Timagami, Ontario.
34. " " " ; Harvard Forest, Petersham, Mass.
35. " " " ; Brattleboro, Vt.
36. " " *silvestris*; Stralsund, Germany.
37. " " " ; Skjaerningsfjell, Ringebii, Norway.
38. " " " ; Great Britain.
39. " " *rigida*; Medford, N. J.
40. " " *mughus;* Central Experimental Farm, Ottawa, Ontario.
41. " *Picea canadensis*; Lake Timagami, Ontario.
42. " " *sitchenis*; Moresby Island, British Columbia.
43. " " " ; Gahrenberg, Germany.
44 and 45. From *Pseudotsuga taxifolia*; Berlin, Germany.
46. From *Larix europea*; Island of Visingsö, Lake Vetter, Sweden.
47. " " *laricina*; Franconia, N. H.
48. " *Thuja plicata*; Vancouver, British Columbia.

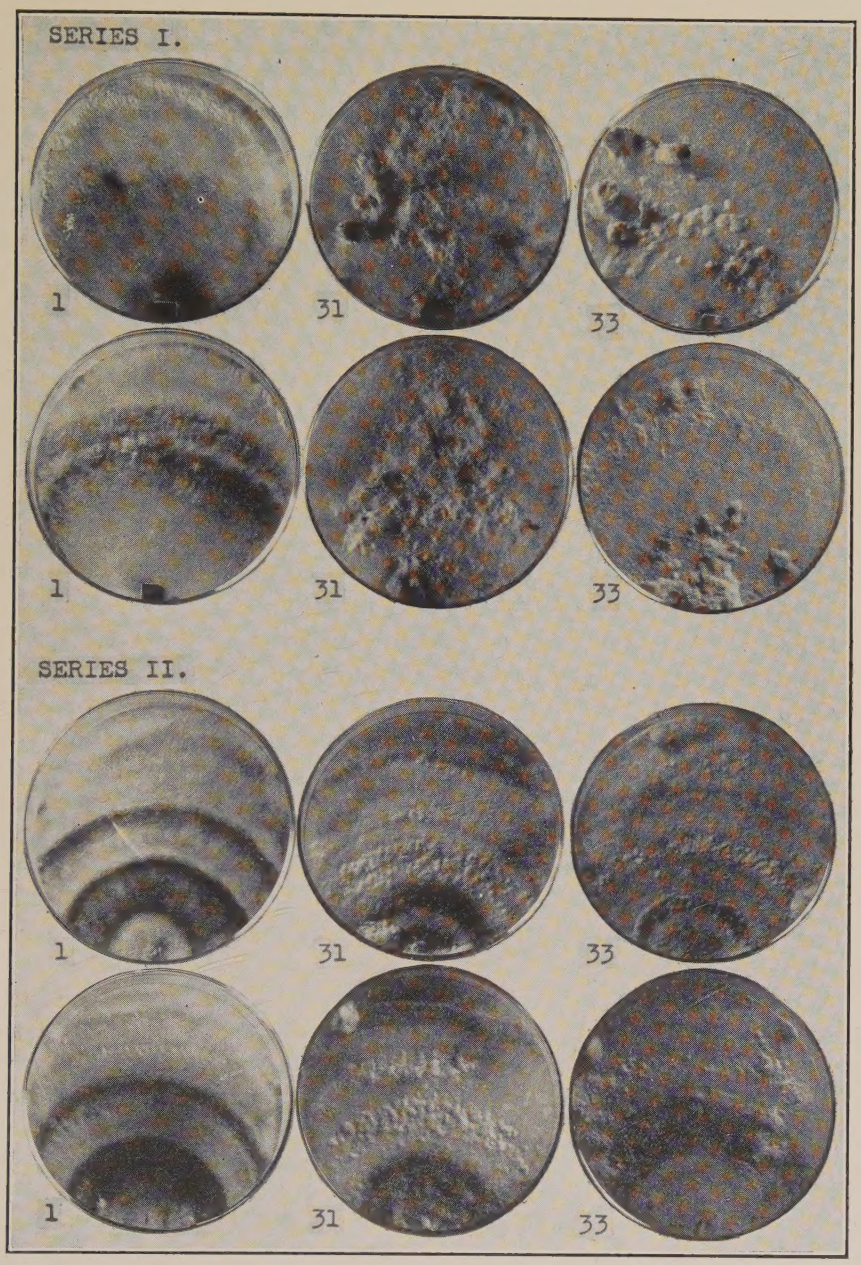

FIG. 1. Cultures showing differences between individual mycelia and between cultures of the same mycelium exposed to different environments, and similarities between parallel cultures of each mycelium. Numerals refer to the numbers given the various mycelia on pages 30 and 32.

49. Host not specified; Timagami Forest Reserve, Ontario.
50. Host not specified; Kyota, Japan.

One isolation of each mycelium was maintained as a stock culture from which all subsequent cultures of that mycelium, except when otherwise noted, were derived.

Cultures of these mycelia were under the writer's observation for periods of from 6 to 20 months, and in no instance was any alteration of cultural characters detected. At the same stage of development, cultures of any given mycelium differed in appearance only when they had been subjected to different environments. This is shown in figure 1, where growth characters of each of 3 representative mycelia are seen to be consistent in duplicate cultures raised under the same conditions (*i.e.*, in the same series) but are not consistent in cultures of the same mycelium in different series. The appearance of a given mycelium cultured in a given environment was determined entirely by the inherent nature of that particular individual mycelium and by the factors of that particular environment; *i.e.*, the cultural history of the material from which transfers were made had no appreciable effect upon the characters of the derived cultures.

Schmitz (14) and Mounce (11) have described and illustrated the line of demarcation that usually develops when different mycelia of *Fomes pinicola* approach each other on the same artificial substratum. An apparently identical reaction occurs between mycelia of *Polyporus schweinitzii*. When isolations from different sources were subcultured on the same slant or plate of nutrient agar, a well-defined dark line about 1 mm. wide usually appeared between them soon after the advancing margins had come in contact. This line did not form between subcultures taken from a single isolation or from similar isolations from any single source, nor did it form when monosporous mycelia were paired with other monosporous mycelia of either the same or different ancestry.

Where not otherwise specified, cultures were made on nutrient agar consisting of 20 g. of malt extract (either Fleischmann's Diamalt or Trommer's Diastasic) and 20 g. of agar per litre of distilled water. Sterilization was effected by autoclaving for 15 minutes at a pressure of 15 pounds. Growth was somewhat more luxuriant and fruiting was more frequent on media made with Diamalt. Trommer's extract was variable, some bottles being caramelized to a much greater extent than others, but the line of demarcation formed between mycelia from different sources was generally darker and more distinct when this brand of nutrient was used. In other respects these two brands of malt extract did not differ perceptibly in their effect on growth characters.

As Fritz (3) has remarked, *Polyporus schweinitzii* is quite sensitive to

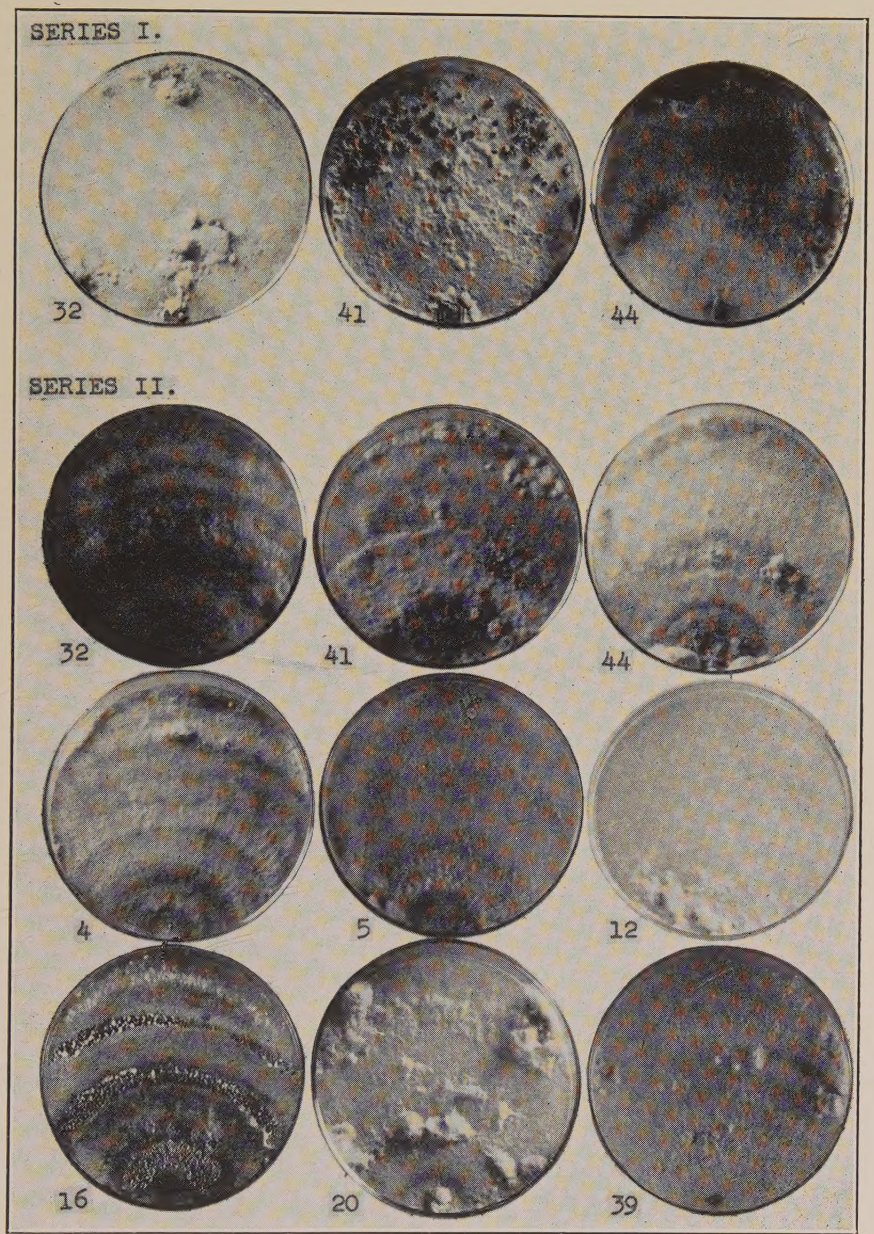

FIG. 2. Cultures showing differences between individual mycelia. Note sporophores in Nos. 16 and 41.

TABLE 1.—*Descriptions of some cultures of representative mycelia*

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
1.	I.	Sienna to olive brown; short pile (occasionally with 1 or 2 indistinct concentric zones of longer and darker pile).		Pale and sparse.	Indistinct. (Fig. 1).
	II.	Dark greenish-brown; uniform and moderately long pile.	Brown; uniform and moderately long pile.	Light red brown to lemon yellow; uniform and moderately long pile.	Quite distinct. (Fig. 1).
2.	I.	Yellow and light brown; fairly even mat.		Pale and sparse.	None.
	II.	Mostly greenish or reddish brown, with some lemon yellow to creamy yellow; regular and moderately long pile (occasionally slightly massed, tufted, or fluffy).			Fairly distinct.
3.	I.	Red brown; numerous very small tufts (some areas with short and fairly regular pile).		Lemon yellow.	Indistinct.
	II.	Light orange brown; fairly regular pile becoming very slightly and uniformly tufted.	Pale to lemon yellow; sparse to moderately long and irregular pile becoming slightly fluffy near edge of culture.		Fairly distinct.
4.	I.	Yellow brown (sometimes with one or two concentric zones of lighter yellow); short, matted pile.		Pale to lemon yellow.	Fairly distinct.
	II.	Olive to greenish or reddish brown; moderately long and uniform pile.			Fairly distinct. (Fig. 2).
5.	I.	Pale lemon yellow to reddish brown; an uneven mat.			Vague.
	II.	Yellow to orange; moderately long and irregular pile (some concentric zones of tufted, compacted, or fluffy growth.			Generally indistinct. (Fig. 2).
7.	II.	Mostly rather pale but with 2 or 3 fairly distinct concentric zones of lemon yellow to light brown; sparse except in regions of darker color, where regular pile is fairly well developed.			Fairly distinct.
12.	II.	Lemon yellow; short and irregular pile with some semicompact or slightly fluffy growth.	Pale and sparse.	Very pale; very sparse.	None. (Fig. 2).
18.	II.	Zones of reddish brown, short, and regular pile alternate concentrically with zones of dark yellow to orange, longer (sometimes slightly fluffy) pile.		Lemon yellow; moderately long and fairly regular pile (sometimes slightly fluffed).	Quite distinct.

TABLE 1.—(*Continued*)

No.	Series	Color and character of growth			Concentric zonation
		Near inoculum	Intermediate region	Farthest from inoculum	
31.	I.	Dark, mottled, yellow brown; irregular, matted pile with frequent fluffy growth.		Pale and sparse.	None. (Fig. 1).
	II.	Greenish to reddish brown; rather long and irregular pile.	Dark creamy yellow; small to medium large rounded tufts.	Pale to yellow green (with some reddish brown); sparse, irregular, or fluffy.	Fairly distinct. (Fig. 1).
42.	I.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with numerous long, cobwebby, lemon yellow to brown wefts or fluffy masses.	None.
	II.	Pale and sparse.	Pale and sparse.	Generally pale and sparse but with zones of loose, creamy wefts and some creamy to yellow green fluffs at edge of culture.	Rather vague.
44.	I.	Pale to yellow to reddish brown; very thin mat with short, sparse, pile (some lemon yellow, fluffy growth at edge of culture).			None. (Fig. 2).
	II.	Dark cream to orange; irregular (sometimes fluffy) pile.	Light to dark yellow green; pile irregular (fluffy in some central areas and near edge of culture).		Indistinct. (Fig. 2).
47.	I.	Yellow brown to reddish brown; short, irregular, pile and occasional small yellow fluffs.	Generally pale and sparse but usually with some pale lemon yellow growth near the edge of the culture.		Vague.
49.	II.	Greenish yellow, orange, and reddish brown; fairly long and regular pile (with a few small tufts).	A narrow zone of creamy, moderately fluffy growth.	Creamy to yellow green; sparse pile with some fairly well developed fluffy growth.	Rather indistinct.

light, growing more slowly and displaying darker and more varied coloration when exposed, even for very short periods, to indirect daylight than when kept in darkness. High humidity stimulates the development of fluffy superficial growth. Temperature, concentration and kind of nutrient, etc., also affect the appearance of cultures. The continuous nature of these environmental variables and the apparently great number of hereditary factors involved make it impracticable to describe in detail all the mycelia studied. Partial descriptions of a few representative mycelia cultured in Petri dishes are presented in table 1, however, to indicate the range and kind of cultural variations encountered during the study. In this table, and in figures 1 and 2, series I consists of cultures stored at a constant temperature of 22°[4] and exposed to electric light, but never to daylight, for a few seconds every 12 hours, while series II consists of cultures stored at room temperature (10° to 25°) and exposed to diffuse daylight for a few seconds every 4 or 5 days. Descriptions of series I were taken on the 19th day and photographs on the 40th day of growth. Descriptions and photographs of series II were taken on the 29th day of growth. From 2 to 20 cultures were made of each mycelium in each series. Cultural characters of each mycelium in each series were consistent in all cases where not otherwise indicated.

From the descriptions in table 1, and from the photographs in figures 1 and 2, it is apparent that differences between mycelia may sometimes be large enough to make difficult the diagnosis of wood decay by cultural methods. Identification may be facilitated by culturing on agar slants in ordinary test tubes and by storing cultures where they will be exposed to daylight of low intensity, since these conditions promote the formation of the more or less leathery mat and hasten the appearance of the "range of yellow tints" (3) typical of this species, but, even under these conditions, occasional mycelia are not readily identifiable on the basis of their cultural characters. The writer has not investigated the microscopic features of the various mycelia to a sufficient extent to determine their reliability as criteria of specific identity. Hasty and superficial observations indicate, however, that differences in microscopic characters are neither so great nor so frequent as are differences in gross cultural characters.

A number of mycelia were noticeably destructive to the agar, in some spots lowering the level of the surface 3 mm. or more below that of the rest of the culture (Fig. 3, 40K). Such lowering usually was confined to the region near the inoculum, but sometimes occurred in other portions of the culture. This destructive effect upon the agar, and the particular region in which destruction was visible, were quite as characteristic of certain mycelia as were the color and character of growth in culture. In cultures

[4] All temperatures recorded in this paper are in °C.

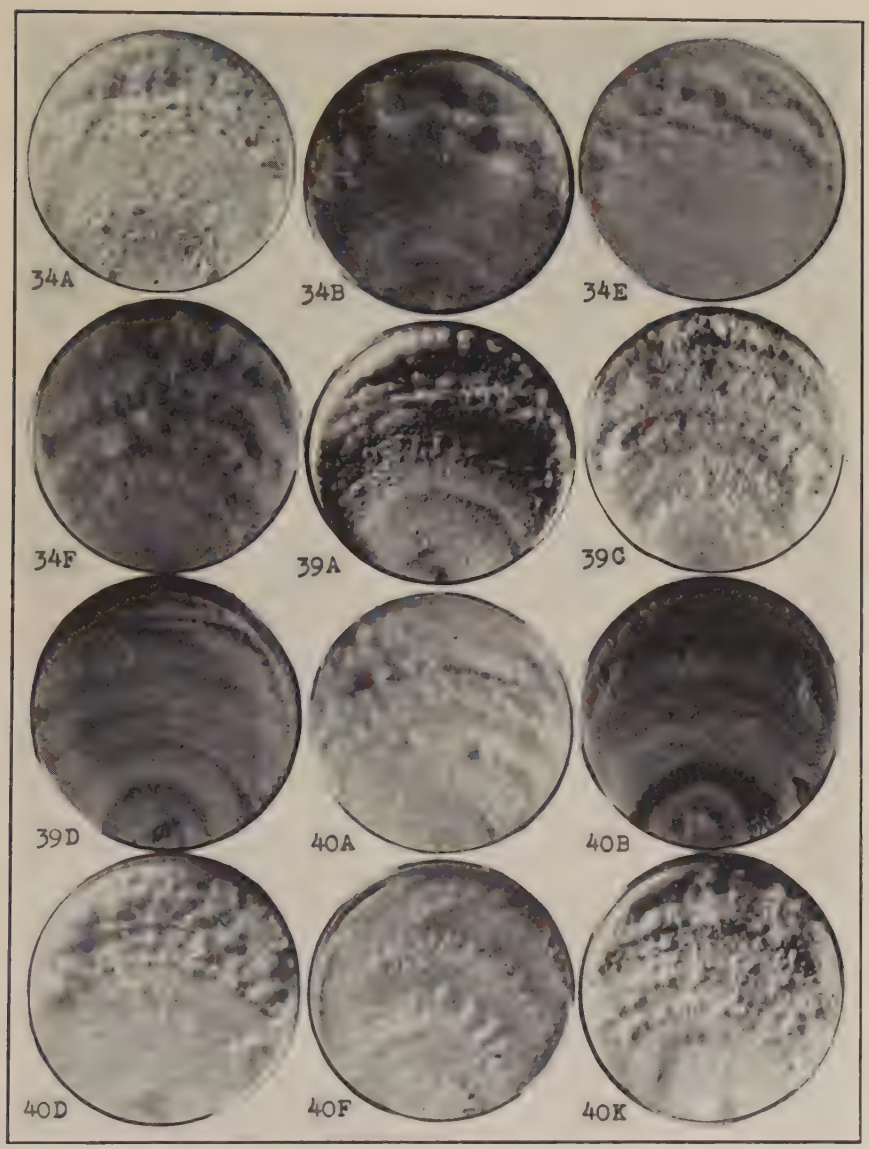

FIG. 3. Cultures showing individual differences between monosporous mycelia. Note sporophores in No. 40B and destruction of agar near inoculum in No. 40K.

of mycelia that did not alter the level of the surface of the substratum the agar retained its original gelatinous consistency until dry, but mycelia that caused any perceptible lowering of the agar surface also rendered the rest of the agar definitely crumbly, even in regions not adjacent to spots of visible destruction. Schmitz (14) has shown that some mycelia of *Fomes pinicola* secrete larger quantities of certain enzymes than are produced by other mycelia of the same species. The phenomenon just described suggests that mycelia of *Polyporus schweinitzii* also differ in enzyme production.

In only one instance during the entire study did repeated isolations from the same source fail to yield identical cultures. Two isolations each of Nos. 23 and 24 were made from a single sporophore. These mycelia differed so markedly in appearance as to be distinguishable from each other during very early stages of development. No. 23 was in no way noteworthy, displaying the usual cultural characters of the species and differing from most of the other mycelia only in relatively minor details. No. 24, however, became established very rapidly at ordinary temperatures and quickly filled the space above the agar slants with a permanently fluffy mass of hyphae. Portions of this mass ranged from a faint lemon yellow to a very light red brown, but most of it remained white throughout the life of the culture. In many respects this individual resembled some of the single-spore cultures described in a subsequent section.

No. 47, the only mycelium found in more than one individual host, was isolated several times from each of 3 wind-thrown larches separated from each other by distances of 20 to 30 feet. Since previous experience had shown that even neighboring hosts of the same species usually were infected by different mycelia, isolations from each of these trees were kept under close observation for about 6 months. At no time during this period were any greater differences detected between them than were to be found between parallel cultures derived from any single mycelium, nor was a line of demarcation formed when they grew together on a common substratum. Fruiting bodies have not been produced by these isolations and the possibility that they represent different mycelia belonging to a definite and perhaps specialized strain, therefore, has not been tested. Their homogeneity suggests, however, that a single mycelium has spread vegetatively to trees near the one originally infected.

RATE OF GROWTH ON NUTRIENT AGAR

During the growth of the cultures in series I, described in the preceding section, the radius of each culture was determined every 12 hours by measuring along the diameter of the Petri dish from the edge of the inoculum to the margin of the colony. Table 2 contains growth data derived from these measurements for 15 representative mycelia. (Nos. 4a and 35a were started

TABLE 2.—*Growth of representative mycelia in series I*

Mycelium No.	Days since establishment of cultures								Diff. between max. and min. on 8th day
	1	2	3	4	5	6	7	8	
	Average radius of mycelium—mm.								*mm.*
1.	11	17	23	28	35	43	51	58	7
2.	10	16	21	27	33	39	47	55	2
3.	10	16	22	29	37	45	53	61	8
4.	10	16	21	28	34	41	48	53	13
4a.	11	17	23	29	34	40	46	54	9
5.	10	16	23	30	37	44	52	60	3
31.	10	16	22	28	36	44	52	60	3
35.	10	15	21	27	33	40	47	55	3
35a.	10	15	21	27	34	40	48	56	3
38.	9	18	28	37	46	55	64	74	3
40.	8	11	14	18	22	25	30	34	13
41.	11	18	25	33	42	51	60	69	4
42.	12	21	30	38	46	55	64	74	6
43.	12	19	27	33	40	50	59	68	2
46.	13	22	30	38	46	54	62	70	2
48.	10	15	21	28	34	42	49	56	4
50.	10	16	21	27	34	41	49	56	6

one week later than the rest of the series, but were otherwise identical in origin and treatment with Nos. 4 and 35, respectively). Averages for each mycelium are based on 4 cultures of that mycelium. The principal difficulty encountered in studies of growth rates in this species was the difference in time required by the various mycelia for establishment on new substrata. In most instances, this difference was correlated with the character of the growth in cultures from which transfers were made. Other factors being equal, establishment was rapid when the transferred material included fluffs of loose aerial hyphae; less so when the aerial growth was compacted or matted; and slowest when the projecting hyphae were sparse and short. Mycelia that were to be transferred for comparative purposes were invariably cultured under the same conditions, but, because of inherent morphological differences, it was impossible to secure the same type of material for transfer from different mycelia. For this reason, the age of each mycelium in table 2 is computed from the time when the 4 cultures of that mycelium attained an average radius of 5 mm. (arbitrarily taken to indicate establishment) instead of from the time the transfers were made. Averages have been calculated, where necessary, by straight-line interpolation between the original semi-daily averages. The close correspondence between Nos. 4 and 4a, and between Nos. 35 and 35a, shows that, within rather wide limits, the

age of the agar is not an important factor, and that the arrangement of the data in this table may therefore be considered legitimate.

Differences between mycelia are clearly apparent in table 2. It is evident that Nos. 38, 41, 42, 43, and 46 have attained a greater radius and are growing more rapidly than the other mycelia, and that the growth rate of No. 40 is relatively low. Relative growth rates of mycelia cultured at higher or lower temperatures than series I were, however, frequently not consistent with the classification suggested by this table. For example, at 27° No. 43 grew more rapidly than No. 42, while at room temperature (Series II) No. 42 grew more rapidly than did No. 43. The cultures of No. 48 included in series II increased their average radius from 8 to 84 mm. in 14 days, while the cultures of No. 50 in the same series increased from 7 to only 65 mm. during the same period: at 27° the growth of No. 50 was appreciably more rapid than was that of No. 48. In every case where 2 mycelia differed significantly in growth rate at the extremes of the temperature range (app. 10° to 27°) to which they were exposed, but not at one or more of the intermediate temperatures, the mycelium that grew relatively rapidly at one extreme grew relatively slowly at the other. These observations indicate a difference between mycelia either in the form of the temperature-growth curve, or in the location of the maximum ordinate of this curve (*i.e.*, the optimum), or both.

Inclusion of the 3 mycelia from spruce in the group of rapidly growing individuals in table 2 is probably the result of coincidence rather than of any host-correlated relationship, since these 3 mycelia differed rather widely from one another in growth rate at other temperatures. Mycelia from Springwater, with one or two exceptions, maintained their relative rates of growth more consistently at the different temperatures to which they were subjected, and resembled each other more closely in absolute growth rates than did mycelia from other sources. This tendency toward similarity, even if not fortuitous, does not necessarily imply the existence of a specialized strain at Springwater. It may indicate only the closer degree of relationship that might logically be expected between isolations from a limited area as compared with isolations from widely separated points.

EFFECT OF ACIDITY ON GROWTH

Nutrient solutions adjusted to 9 different acidities were prepared by mixing 3 parts of 3 per cent Trommer's malt solution with 2 parts of KH phthalate buffers compounded according to the formulas of Clark and Lubs (2). The buffered solutions were placed in 38 × 200-mm. test tubes (app. 65 cc. of solution per tube) and autoclaved 15 min. at 15 lbs. pressure. The acidity of a sample from each lot was then determined potentiometrically, and the remaining tubes were inoculated with various representative my-

celia. One culture at each pH was made of each mycelium, except No. 1, which was cultured in duplicate. Twenty-three days after inoculation the depth of the mycelium and the acidity of the medium in each tube were determined (Table 3). Since in no case did the change in pH during the

TABLE 3.—*Effect of acidity on growth of mycelium of Polyporus schweinitzii*

Initial pH of buffered nutrient	Mycelium No.									Final pH
	1	1	8	31	40	42	47	49	50	
	Depth of mycelium—mm.									
2.5	9	10	5	5	8	8	6	6	15	2.6
3.0	18	18	13	12	15	15	10	10	28	3.0 to 3.2
3.5	26	27	28	30	22	34	16	25	45	3.6 to 3.7
4.0	40	43	41	44	45	46	37	39	52	4.2
4.3	42	44	50	45	46	52	50	42	52	4.5
4.7	44	46	55	39	40	50	43	42	55	4.8 to 4.9
5.1	43	43	53	36	7	48	39	46	55	5.2
5.4	22	21	28	17	9	20	20	34	33	5.4 to 5.6
5.7	10	10	10	7	4	11	7	12	15	5.6 to 5.9

growth of the fungus exceed 0.2, and since the 2 cultures of No. 1 at each acidity were reasonably consistent, the data in this table are considered approximately indicative of the effect of acidity on the growth of these mycelia. It will be seen that in every instance the optimum pH for growth was greater than 4.0 and less than 5.4, and in most cases probably lay between 4.2 and 5.0. Within these limits, however, the acidity permitting maximum growth was somewhat different for different mycelia. For example, Nos. 31 and 40 obviously made their best growth in more acid media than did Nos. 49 and 50.

RATE OF DECAY OF WOOD BLOCKS IN VITRO

Thirty-two white pine blocks (all from the same tree) approximately $\frac{7}{8}'' \times \frac{7}{8}'' \times 4''$ were air-dried in the laboratory for several weeks, given individual numbers, weighed to the nearest half-gram, placed in 38×200-mm. test tubes half full of water, and autoclaved 30 min. at a pressure of 15 lbs. They were then transferred to similar tubes, each of which contained a pure culture of the fungus growing actively in about 25 cc. of 2 per cent malt-extract solution, and stored in darkness at 22°. Two months later an additional 30 cc. of sterile distilled water was added to each tube. After a total incubation period of nearly 9 months the blocks were removed from the tubes, air-dried for 3 weeks, and reweighed. Average losses in weight, expressed as percentages of the original air-dry weights, were as follows:

Heartwood	
9 blocks inoculated with No. 1	4.9 ± 0.7%
7 " " " " 39	3.7 ± 0.7%
Difference	1.2 ± 1.0%
Sapwood	
9 blocks inoculated with No. 1	17.7 ± 1.0%
7 " " " " 39	24.9 ± 2.7%
Difference	7.2 ± 2.4%

The difference between the 2 sets of heartwood samples cannot be considered significant, since it is only slightly greater than its probable error.[5] The difference between the 2 sets of sapwood samples, however, is 3 times its probable error, and in spite of the small basis it may, therefore, be concluded that there probably is a real difference in the ability of these 2 mycelia to attack sapwood under the conditions of this experiment. Schmitz (14) presents evidence of similar differences between mycelia of *Fomes pinicola*. In his studies, as in the present instance, the relative order of destructiveness in heartwood is not the same as that in sapwood.

SPOROPHORES IN ARTIFICIAL CULTURE

Eleven mycelia, including isolations from both sporophore tissue and decayed wood, fruited *in vitro* during the course of these studies. Nine of the 11 fruited under conditions to which most or all of the 39 sterile mycelia also were exposed, while the remaining 2 produced sporophores only under conditions to which less than half of the other mycelia were subjected. A few of the fertile mycelia fruited commonly: in others, fruiting was rare or sporadic.

In the series of wood-block cultures described in the preceding section, No. 39 formed sporophores consistently on sapwood but not on heartwood. It also fruited several times on white-pine sawdust and at the surface of cultures growing in a 2 per cent solution of malt extract. The only other mycelium cultured on these media was No. 1, which failed to fruit on any of them.

On Trommer's malt agar, Nos. 39 and 50 produced one sporophore each. Nos. 23, 30, 34, and 41 occasionally formed a few sporophores, and No. 40 fruited rather commonly. On this medium, sporophores usually appeared only in rather old cultures. Fruiting, therefore, might have been less sporadic had it been possible to retain the cultures longer.

Diamalt agar was apparently a somewhat better medium for sporophore

[5] Standard deviation $= \sqrt{\frac{\Sigma(fd^2)}{n-1}}$

Probable error of average $= \frac{0.6745\ \text{S.D.}}{\sqrt{n}}$

Probable error of difference $= \sqrt{\text{P.E.}_a{}^2 + \text{P.E.}_b{}^2}$

production. No. 1 fruited rarely; Nos. 9, 13, 23, and 41 fruited occasionally; and No. 16 fruited fairly regularly on this medium. No. 34 never produced sporophores in test-tube cultures but did so invariably in Petri dishes except when the layer of agar was quite thin. The most consistent and prolific fruiting encountered during the entire study was afforded by No. 40. This mycelium commonly produced sporophores in test-tube cultures: in Petri dishes, sporophore rudiments appeared soon after the surface of the agar was overgrown and functioning sporophores were often present in cultures only 3 weeks old.

The largest sporophores were those of Nos. 39 and 50, which averaged 1 cm. in diameter and ranged from 5 to 15 mm. in thickness: the smallest were those of No. 40, which averaged 3 mm. in diameter and 2 mm. in thickness. Color varied from yellow (Nos. 34 and 40) to reddish brown (No. 50) or dark brown (No. 39). Neither stipes nor recognizable pilei were present in any instance. Sporophores that developed above the culture medium consisted principally of short spines (produced by the majority of the fertile mycelia), small and disarticulated lamellae (Nos. 34 and 40), or longer hydnoid processes, frequently once- or twice-dichotomized (formed only by No. 39). Pores occurred infrequently, and then only in sporophores borne laterally, as on wood blocks and vertical agar slants, or vertically, as in inverted Petri dishes. Average pore dimater varied from less than 0.5 mm. in No. 40 to slightly more than 1 mm. in No. 39. Small pores were approximately circular but the larger ones were quite angular. Young fruiting bodies that had recently become spiny or lamellate could sometimes be induced to form pores by reversing their orientation with respect to gravity. Neither sporophore production nor pore formation appeared to depend on exposure to daylight. The microscopic structure of hymenia in artificial culture did not differ essentially from that of hymenia developed under natural conditions. Sporophores formed by different mycelia displayed some microscopic dissimilarities—for example, cystidia of No. 34 were relatively large and sometimes terminally swollen, while those of No. 40 were smaller and of more nearly uniform diameter throughout. These variations, however, were no greater than those occurring normally in "wild" sporophores. It seems probable that the well-known variability of the sporophores of many species of Polyporaceae under natural conditions (9, 15) is simply another example of differences between individuals.

MONOSPOROUS AND POLYSPOROUS MYCELIA

Basidiospores of *Polyporus schweinitzii* are so small and fragile that it is extremely difficult to secure uninjured single spores by Hanna's dry needle method (4), even with micromanipulation apparatus. Progressive dilution of material from spore prints is much less laborious, but the serviceability

of this method is impaired by the persistence with which many of the spores remain aggregated in small groups throughout the process of dilution. The procedure devised by Mounce (11),[6] however, proved quite satisfactory and in a slightly modified form was followed in obtaining all single-spore cultures except those from No. 39, which were secured by dilution. Polysporous cultures were obtained by scraping an uncontaminated spore print with a sterile spear-pointed needle and planting the material thus obtained on a slant of nutrient agar. Both monosporous and polysporous cultures were derived in all cases from spores produced by pure cultures of the fungus.

Fresh spores germinated readily, and numerous monosporous cultures were obtained from Nos. 34, 39, 40, and 50. These monosporous cultures resembled the stock mycelia in many respects. The mat developed on the surface of the agar seemed, however, generally thinner and more delicate, and superficial growth was often much fluffier. The following, cultured under the same conditions as series II, are fairly representative (Figure 3):

No. 34A.[7] Orange brown and rather irregular pile with numerous short, fluffy, pale lemon yellow tufts (Fig. 3).

No. 34B. Growth sparse and pale near inoculum; becoming pale yellow and loosely fluffed at other side of culture (Fig. 3).

No. 34D. Orange to dark olive brown pile near inoculum; pale lemon and loosely tangled fluff at other side of culture.

No. 39A. Reddish brown near inoculum; reddish brown pile with numerous short tufts and fluffy cream-color masses at other side of culture (Fig. 3).

No. 39B. Reddish brown near inoculum; a mixture of light orange and dark cream at far side of culture.

No. 39C. Zones of pale, sparse growth alternate with zones of orange pile near inoculum and with zones of cream-color fluffy growth at far side of culture (Fig. 3).

No. 40A. Growth near inoculum mostly pale and scantily fluffy; rest of culture consists of light to dark yellow fluff (Fig. 3).

No. 40B. Sparse and pale near inoculum, with some destruction of agar; rest of culture dark lemon yellow to olive or red brown (Fig. 3).

[6] "A sporophore was removed from a culture with sterile forceps and quickly fastened to the cover of a sterile Petri dish by means of a drop of melted agar. Then the cover was removed from a Petri dish containing lactose gelatine, the cover bearing the sporophore was substituted, and slowly rotated. Then the cover was replaced and as many Petri dishes as desired were inoculated in this way. The plates were kept at room temperature for from four to five days. By that time, the germinating spores appeared as tiny depressions in the surface of the clear gelatine. A circle was drawn around each mycelium, it was examined under the microscope, and if it proved to be monosporous, and if there were no other spores within the circle, it, surrounded by a small square of gelatine, was removed with a sterile spear-shaped needle, placed on a potato-dextrose or malt agar slant, and grown at room temperature."

[7] Numeral refers to parent mycelium—*e.g.*, No. 34A is the first monosporous mycelium secured from No. 34.

No. 40C. Mostly sparse and pale near inoculum; rest of culture raggedly fluffy and pale yellow to dark creamy yellow or light orange.

No. 40F. Zones of sparse and pale growth alternate with zones of short, lemon yellow tufts (Fig. 3).

No. 40K. Most of agar destroyed in immediate vicinity of inoculum; rest of culture consists of light to dark yellow fluff (Fig. 3).

From these descriptions, and from table 4 and figure 3, it will be seen

TABLE 4.—*Growth of representative mycelia of both monosporous and natural origin (Series II)*

Mycelium No.[a]	Age of cultures—days				Diff. between max. and min. on 16th day
	7	11	16	21	
	Average radius—mm.				*mm.*
1.	15	27	49	77	2
3.	2	13	41	69	4
4.	18	33	64	82	4
34.	6	17	41	63	3
34A.	22	39	65	[b]	0
34C.	18	36	67	[b]	8
34D.	22	40	68	[b]	3
34F.	16	28	48	63	4
39.	8	25	56	80	0
39A.	9	27	55	79	2
39C.	12	26	54	74	2
39D.	10	23	38	54	8
40A.	12	32	63	83	2
40B.	13	31	60	81	7
40E.	12	32	69	[b]	2
40F.	13	30	56	76	7
40K.	11	29	62	[b]	1
41.	13	32	63	82	7
43.	6	19	39	62	9
46.	7	23	56	90	2
48.	8	24	51	84	6

[a] Data for each mycelium are based on 2 cultures.

[b] Entire surface of culture overgrown (radius greater than 90 mm.).

that differences between monosporous mycelia are of the same general type and of about the same order of magnitude as those between isolations secured from different sources in the field. Cultural differences are ill-adapted to quantitative expression, and an exact comparison of the extent of variability in the different groups of monosporous mycelia of common descent is, therefore, impracticable; in general, however, it may be said that mycelia having the same immediate ancestry appeared to differ less than did those of more diverse descent. Individual differences were apparent, however, even be-

tween mycelia descended from the same sporophore. When cultured under the same conditions, hyphal-tip transfers from opposite sides of a given monosporous colony were culturally identical.

Twenty-four of the 41 monosporous mycelia secured from No. 40 produced sporophores. From one of these fertile monosporous mycelia (No. 40L) 33 single-spore cultures were made, of which 30 produced sporophores. These third-generation cultures resembled each other very closely, much more so than did those of the preceding generation, but could not be considered identical in appearance. The only other sporophores that appeared in either monosporous or polysporous cultures were developed by a few descendants of Nos. 39 and 50. Some of these were presumptively of single-spore origin, but a few of the supposedly monosporous cultures (including the fertile ones) derived from No. 39 showed striking similarity to polysporous cultures from the same mycelium: In view of the defects of the dilution method (used with spores from this mycelium only) the monosporous origin of these cultures is doubtful.

Polysporous cultures from Nos. 34 and 40 were similar in general appearance to isolations secured in the field, but polysporous cultures from Nos. 39 and 50 displayed none of the gross characteristics of *Polyporus schweinitzii*. The hyphae in the latter two were hyaline, giving cultures on malt agar a dirty grey appearance, and were almost entirely confined to the medium within 1 mm. of the surface of the agar slants. Polysporous cultures from No. 39 produced a few short aerial hyphae that looked like very fine cotton lint sparsely sprinkled on the surface of the agar. Those from No. 50 produced no aerial growth. In other respects these cultures were indistinguishable from one another. After repeated transfers, several of the polysporous cultures from No. 39 developed thin, irregular, dark reddish brown mats on the surface of the agar slants: such cultures invariably produced sporophores.

DISCUSSION

Morphological or cultural characters and more purely physiological qualities are not necessarily correlated (1). Nevertheless, the wide range of variability apparent between the cultures from Springwater, a range almost as great as that found between cultures from widely separated points and unlike hosts throughout the Northern Hemisphere, indicates that damage in this locality cannot now be attributed definitely to infection by a race or strain of special virulence. The planting stock used in the infected areas at Springwater came from nurseries in which seedlings from European nurseries had been transplanted, and other plantings in the immediate neighborhood have been made with stock of European origin, but the large number of different mycelia isolated from the infected trees precludes the possibility of infection having originated entirely by vegetative spread of the organism

from one or a few foci in nursery beds. Fruiting bodies of *Polyporus schweinitzii* have been rare in these plantings until quite recently.[8] It seems unlikely, therefore, that a few original infections by a virulent strain have been spore-propagated, with consequent morphological diversification of the organism, to a sufficient extent to account for the large and varied population of this species now present in these areas. Wean[9] has shown that infection may occur in very young trees, but his studies also indicate rather definitely that parasitic activity of this fungus is favored by certain conditions, such as those at Springwater, which are suboptimum for white pine. Pending the completion of inoculation studies in the field it must accordingly be concluded that the *Polyporus schweinitzii* epiphytotic in this locality probably is attributable to edaphic factors that have permitted extensive infection by native representatives of the species.

The possibilities of future specialization created by the wide variability exhibited by this fungus must not be overlooked. It may be assumed that the majority of the individuals constituting this species are now adapted to a quasi-parasitic life in the basal heartwood of older trees, but extensive infection of younger trees growing under more or less unnatural conditions might conceivably result in the gradual selection of lines best adapted to such existence and a consequent increase in the average virulence of the species to planted trees even on good sites. The likelihood of such a change and the time necessary for it to occur must remain problematical, but the immediate values involved are great enough to justify the development and adoption of preventive and control measures.

Variability of the type described in this paper is by no means peculiar to the Polyporaceae. Recent pathological literature contains numerous references to similar instances of intraspecific variability in widely separated species of fungi. The work of Magie (10) on *Coccomyces hiemalis,* Johnson and Valleau (7) on *Thielaviopsis basicola,* Palmiter (12) on *Venturia inaequalis,* and Hansen and Smith (5) on *Botrytis cinerea* may be cited as random examples. All of these workers observed differences in cultural appearance between isolations, and in some cases also found differences in such characters as growth rate, spore production, size and shape of conidia, pathogenicity, reaction to toxic substances, and reaction to acidity of the medium. None of these differences were correlated with the host or locality from which the isolations were made. Palmiter's (12) statement seems generally applicable to all of the above-mentioned species: "No two of the 36 isolations studied appeared exactly alike when grown under standardized conditions, yet duplicate cultures could easily be recognized. . . . *V. inaequalis*

[8] York, H. H. A study of resinosis and root rot in forest plantings of *Pinus strobus* and *P. resinosa.* Unpublished manuscript.

[9] Wean, R. E. The parasitism of *Polyporus schweinitzii* on seedling *Pinus strobus.* Unpublished manuscript.

is not a homogeneous species or one composed of a few well-defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters.'' Research on such highly heterozygous species can be of maximum usefulness only when the sampling errors inseparable from studies of varied populations have been reduced by the use of a large and representative basis. Small bases may be sufficient in species characterized by slight variability, but in most instances a high degree of variability must be assumed until investigations of numerous isolates have shown the species to be essentially uniform for the character in question. It seems probable that discrepancies between quantitative data secured by competent workers often may be charged directly to the basing of studies on only one or a few individuals from species in which wide variation may exist. For example, Lagerberg and Melin,[10] Robak (13), and Weis and Nielson (16) disagree rather widely as to the optimum pH for growth of *Fomes annosus* (Fr.) Cke. in artificial culture. This disagreement may be the result of differences in experimental conditions, but the observations of Palmiter (12) and the present writer suggest that it is caused by differences between the individual mycelia used in the various studies.

The term ''strain'' often is employed by pathologists to designate a single original isolation and its subcultures (with the exception of saltants, etc.). Its use in this particular connection is unfortunate, since it frequently leads the reader to an erroneous conception of the relationships involved, even when it does not indicate a misunderstanding on the part of the user. ''Strain'' suggests a racial grouping[11] of genotypically distinct individuals that are homozygous for one or more physiologic or cultural characters serving to distinguish them from other members of the species. In many instances the term is, of course, correctly used, but it is not applicable to any one of the different mycelia of *Polyporus schweinitzii* herein described. Genetic irregularities and the frequent occurrence of asexual reproduction obscure the individualization of fungi, but in this group, as in any other group of organisms, individuals are the concrete units into which such abstract conceptions as strains and species are ultimately resolvable. The situation is made more complex but is not fundamentally altered by such anomalous phenomena as mixochimaeras (heterocaryotic cells), ''discontinuous variations'', and physical discontinuity of individual genotypes. For practical purposes, separated fragments of an organism or new colonies

[10] Not seen by the present writer; *fide* Weis and Nielson (16).

[11] ''When the isolates are compared on various culture media and under different environmental conditions they fall into a number of groups each of which contains isolates which are alike in their cultural characters and cultural behaviour. Each of these groups constitutes a Strain or Race, and equates with Lotsy's 'Jordanon'. . . . Cultural races may be equated with biological races in the more strict sense.'' Brierley (1).

that have originated from accessory spores of the same genotype can be accorded no greater claim to separate individual ranking in Nature than *in vitro*.[12] Brierley's isolates, then, do not necessarily represent different individuals in all cases, although for the sake of convenience they must often be considered to do so. "Mycelium" has been used throughout the present paper in a sense that the writer considers equivalent to "clone". "Genotype" or, less accurately, "individual", might be similarly used. Choice of terms must frequently depend, as in the present instance, on convenience of expression and on the extent to which relationships within the species in question are known. Whatever the terminology, the essential fact remains that some species of fungi, of which *Fomes pinicola* (11) and *Polyporus schweinitzii* may be cited as examples among the Polyporaceae, are made up of individuals that differ from one another in many respects, and that investigations of such species must, therefore, include a sufficient number of individuals to permit an approximate determination of the specific average and range of variability.

SUMMARY

Cultural studies of 50 different mycelia of *Polyporus schweinitzii*, secured from various coniferous hosts and widely separated points throughout the Northern Hemisphere, show that this species is made up of individuals that differ rather widely from one another in appearance in culture, production of sporophores, growth rate on nutrient agar, reaction to acidity, and apparently also in ability to cause decay of wood. Individual differences also were found between monosporous mycelia secured from spores produced by pure cultures of the fungus. There was no evidence of the occurrence of local or host-specialized strains within this species, and it therefore seems probable that the serious damage which the fungus is causing to planted white pine near Springwater, N. Y., is attributable to site conditions rather than to any special virulence on the part of the fungus in these plantings. The differences shown to exist between individuals of this species are comparable to those demonstrated by Mounce (11) for *Fomes pinicola*, and indicate that research on such variable species can produce generally applicable results only when a large number of individuals are studied.

DIVISION OF FOREST PATHOLOGY, BUREAU OF PLANT INDUSTRY,
U. S. DEPT. OF AGRICULTURE, PORTLAND, OREGON

LITERATURE CITED

1. BRIERLEY, W. B. Biological races in fungi and their significance in evolution. Ann. Appl. Biol. 18: 420–434. 1931.

[12] "Each separate pure culture made by direct isolation from fresh material, whether a number of cultures are made from a single lesion or from one or more host plants, I term an Isolate. . . . Each isolate is an individual line and sub-cultures are merely duplicates of that isolate or line. The isolate is the nearest equivalent to Lotsy's 'species.'" Brierley (1).

2. CLARK, W. M. The determination of hydrogen ions. 3d ed. 717 pp. Williams and Wilkins Co., Baltimore. 1928.
3. FRITZ, C. W. Cultural criteria for the distinction of wood-destroying fungi. Roy. Soc. Can. Trans. Ser. 3. 17 (Sect. V): 191–288. 1923.
4. HANNA, W. F. The dry-needle method of making monosporous cultures of Hymenomycetes and other fungi. Ann. Bot. [London] 38: 791–795. 1924.
5. HANSEN, H. N., and R. E. SMITH. The mechanism of variation in imperfect fungi: *Botrytis cinerea*. Phytopath. 22: 953–964. 1932.
6. HEDGCOCK, G. G., G. F. GRAVATT, and R. P. MARSHALL. *Polyporus schweinitzii* Fr. on Douglas fir in the eastern United States. Phytopath. 15: 568–569. 1925.
7. JOHNSON, E. M., and W. D. VALLEAU. Cultural variations of *Thielaviopsis basicola*. Phytopath. 25: 1011–1018. 1935.
8. LONG, W. H., and R. M. HARSCH. Pure cultures of wood-rotting fungi on artificial media. Jour. Ag. Res. [U. S.] 12: 33–82. 1918.
9. LOWE, J. L. The Polyporaceae of New York State. (Pileate species). New York State Col. Forestry. Syracuse Univ. Tech. Pub. 41. (Bull. 6 (1-b)). 1934.
10. MAGIE, R. O. Variability of monosporic cultures of *Coccomyces hiemalis*. Phytopath. 25: 131–159. 1935.
11. MOUNCE, I. Studies in forest pathology. II. The biology of *Fomes pinicola* (Sw.) Cooke. Canada Dept. Agr. Bull. (n.s.) 111. 1929.
12. PALMITER, D. H. Variability in monoconidial cultures of *Venturia inaequalis*. Phytopath. 24: 22–47. 1934.
13. ROBAK, H. On the growth of three wood-destroying Polyporeae in relation to the hydrogen-ion concentration of the substratum. Svensk. Bot. Tidskr. 27: 56–76. 1933. [Abstract in Rev. Appl. Mycol. 12: 543–544. 1933. Original not seen.]
14. SCHMITZ, H. Studies in wood decay. V. Physiological specialization in *Fomes pinicola* Fr. Amer. Jour. Bot. 12: 163–177. 1925.
15. SHOPE, P. F. The Polyporaceae of Colorado. Ann. Missouri Bot. Gard. 18: 287–456. 1931.
16. WEIS, F., and N. NIELSON. Nogle Undersøgelser om Rodfordaerversvampen (*Polyporus radiciperda*). [Some investigations of the root-destroying fungus—*Polyporus radiciperda*]. Dansk. Skovfor. Tidsskr. 12: 214–225. 1927. [Abstract in Rev. Appl. Mycol. 7: 551. 1928. Original not seen.]